ANALYSIS OF CATECHINS AND CAFFEINE IN SOME BLISTER BLIGHT RESISTANT & SUSCEPTIBLE TEA CULTIVARS

By

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DECLARATION

I here by declaration that the work reported in the project report was exclusively carried out by me, under supervision of Dr. I.S.B. Abesinghe and Mrs. I. Wickramasinghe. Any part of this project report has not been submitted earlier or concurrently for same or any other degree.

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DEDICATED
TO
MY LOVING PARENTS

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ABSTRACT

Blister blight, a leaf disease caused by the fungus *Exobasidium vexans* Massee, is an economically important disease in tea lands. This disease cause heavy crop losses in the cultivation of tea.

Tea Research Institute has developed *Exobasidium vexans* resistant and susceptible cultivars using conventional plant breeding programme. The study was carried out at TRI, using *Exobasidium vexans* resistant & susceptible selection as determined by the Pathology division from phase I & II stage of the conventional plant breeding programme in order to confirm the possibility of using chemical markers to detect blister blight resistant cultivars.

Nine selections from phase II and seven selections from phase I were selected. These selections included both blister blight resistant & susceptible cultivars, from the field No.12, St.Coombs estate, Thalawakelle. Extraction of the samples for the analysis of individual catechins was done by maceration of samples in 70%(v/v) boiling methanol. Analysis of caffeine and catechins were carried out using high performance liquid chromatography (HPLC).

The results of phase I, revealed that there is no significant correlation between the Epigallocatechin (EGC) content in the tea plant with the blister blight resistance. The results from phase I also revealed that there may be possibility of using Epicatechin (EC) levels together with Epigallocatechin gallate(EGCG) levels in the tea plant to select blister blight resistant & susceptible tea varieties.

According to the results from phase II, EGC content & ECG contents of phase II selections had no correlation to blister blight disease resistance. The EC & EGCG contents of phase II selections indicated that there could be a correlation between EC & EGCG contents of the tea plant and blister blight leaf disease resistance as in the case of phase I selections.

Results obtained from this study confirmed the previous work carried out at TRI, in order to use chemical compounds as an indicator for the blister blight resistance & susceptibility.

Further investigations need to be carried out to confirm the possibility of using chemical markers to detect blister blight resistant varieties of an early stages of the breeding programme.

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ABBREVIATION

C	Catechin
^{0}C	Centigrade
Cu	Copper
EBI	Ergosterol-biosynthesis inhibitors
EC	Epicatechin
ECG	Epicatechin gallate
EDTA	Ethylene Diamine Tettra Acetic acid
Eg	Example
EGC	Epigallocatechin
EGCG	Epigallocatechin gallate
^{0}F	Farenheight
Ha	Hectare
HPLC	High Performance Liquid Chromatography
i.e.	That is
ISO	International Organization for Standardization
OH	Hydroxyl
µm	micrometer
mm	millimeter
PPO	Polyphenol Oxidase
TRI	Tea Research Institute
Var	Variety

CHAPTER 01

1. INTRODUCTION

Tea (*Camellia sinensis*)(L).O. Kuntze is the most popular non alcoholic beverage next to water. This perennial woody plant comes under two main verities i.e. *Camellia sinensis* variety *sinensis* and *Camellia sinensis* verity *assamica*. The tender shoots of tea, comprising two or three of the top most immature leaves and the bud, referred as the 'flush' are harvested or plucked for processing.

1.1. HISTORICAL BACKGROUND OF TEA

Emperor Shen Nung of China discovered tea and he claimed that tea was able to detoxify 72 kind of poisons (Teranishi et al., 1995). The spread of tea from China to other parts of the world is said to have commenced as early as 221 B.C. with the migration of minority nationalities from China to other countries. These migrations were the result of incessant internal wars which prevailed at that time. Then it was gradually spreaded from its' peripheral areas via over land and sea routes. Finally tea became very popular in the both east and west and the present tradition of the afternoon tea became established, particularly as a British habit. With the rapidly growing popularity of tea, cultivation of tea was introduced to several other countries.

Tea had been introduce to Sri Lanka from India as early as 1839, but attempts to propagate it on a commercial scale were not successful until 1886, an enterprising Scotsman, James Taylor, demonstrated that tea could be grown and processed profitably (Teranishi et al., 1995; Wickramasinghe, 1978). Since then time tea industry has developed rapidly and at present approximately 200000 hectares(ha) of land are tea under planted (Teranishi et al., 1995).

1.2. BOTANY OF TEA PLANT

In 1753, in the binomial system originated by Linnaeus, the tea plant was classified as *Thea sinensis*. Many synonyms have been given, but now it is generally accepted that the tea plant is classified in the family Theaceae and in *Camellia* species.

Commercially cultivated tea consist of natural hybrids of 3 main types viz *Camellia sinensis* var *sinensis* for small leaves China plants, *Camellia sinensis* var *assamica* for long leaves Assam plants and *Camellia assamica* var *lasiocalyx* for Southern form.

Tea is a perennial woody plant & it is kept as an ever green shrub by pruning. The leaves are epical in shape. The flowers are white with yellow anther similar to wild rose. The tea fruit contains 3 seeds. When hand picked only the leaf bud and the 1st two leaves are plucked from the plant.

Under natural condition, tea plant could grow into a tree about 20 feet high. But in commercial cultivation its growth has to be modified into a low spreading bush in order to produce large amount of leaf at frequent intervals.

1.3. CHEMSTRY OF TEA

Fresh tea flush makes up the raw material, which is processed to black, green, or other forms of tea. Fresh tea leaves contain many chemical compounds, which determine the quality of the processed tea.

Polyphenols which represent 25 – 35 % of dry matter of fresh tea leaves are the most important compounds present in tea which are responsible to the characteristic properties of tea (Harbowy and Balentine, 1997). The major fraction of tea polyphenols is flavanol that include mainly (-)–Epicatechin(EC), (-)–Epicatechin Gallate(ECG), (-) – Epigallocatechin(EGC), (-) – Epigallocatechin Gallate(EGCG), (+) – Catechin(C) and (+) – Gallocatechin(GC) (Sanderson, 1972; Wickramasinghe, 1978).

90 – 95 % of the above flavanols undergo enzymatic oxidation during the processing of black tea to produce theaflavins and thearubigins, which determine the brightness, color, strength and mouth feel of the infusion (Sandrson, 1972.; Wickramasinghe, 1978). Theogallin and theaflavin play an important part in determining quality of made tea. Theogallin, a polyphenolic compound which accounts for about 1% of the dry weight of tea. Theanine, an amino acid which contributes of 0.85-1.5% of the dry weight. Both of these compounds are unique to tea and have not been found in any other plant.

Theogallin is a "depside" formed by the combination of two acids i.e quinic acid and gallic acid which are chemically quite different to the catechins and theaflavins, and does not undergo any marked quantitative changes during processing.

Theanine is the major amino acid of tea, and its concentration is high in the best grades of green tea. It is establishes that quality of green tea is depends on theanine content.

Caffeine is also an important compound present in tea leaves. Which makes a significant contribution to the tea quality. The quantity of caffeine that infuses into a brew is determined by infusion time and by leaf style.

Table 1.1: Chemical composition of green tea leaf

Component	*Amount % (dry weight)*
Total ploypheno1s	30
Caffeine	3-4
Amino acid	¬4
Simple carbohydrates	¬4
Organic acid	¬0.5
Polysaccharides	¬13
Proteins	¬15
Ash	¬5
Cellulose	¬7
Lignin	¬6
Lipids	¬3
Pigments	¬0.5
Volatile substances	0.01-0.02

Source: (Hilton, 1972).

1.4. BLISTER BLIGHT LEAF DISEASE & THE TEA PLANT

Disease of tea leaves are naturally fallen into 3 groups (Hainsworth; 1952).

1. Endemic and permanent disease
   eg. Black rots, pink disease, thread blights.

2. Epidemic and transient disease
   eg. Blister blight, Copper blight

3. Minor and Secondary diseases
   eg. Brown blight, grey blight, sooty moulds, cercosprolla

Out of these diseases blister blight is a leaf disease, cause by *Exobasidium vexans* and it was first reported in Assam in 1868.It was not until 40 years later that it was detected in Darjeeling, India. In the 1930s it appeared in Cambodia but the disease was not spread to South India and Sri Lanka until 1946 It appears simultaneously in both countries (Arulpragasam, 1992). This primary leaf disease affects tender shoots and succulent stems. Quantitative losses in yield due to blister attack are enormous; and these losses are compounded because blister afflicted leaves produce flaky teas thereby affecting the quality of tea (Banerjee, 1993).

The blister blight spores are small and wind borne and are very susceptible to high light intensity and desiccation. The fungus causing the disease is an obligate parasite incapable of getting its' nourishment from dead matter, and so far as is known able to get it only from living tea bushes. No alternate hosts have been found so far. The most important characteristics of the disease is that it can infect only young and succulent leaves and stems and does not affect the mature leaves. As the leaves become older they develop resistance to infection (Arulpragasam, 1992).

The first visible symptom of the disease is a pale yellow or lime green spot which is more translucent than the rest of the leaf. The spots may sometimes be pink, red or brown depending on the reaction of the different tea clones to the infection (Arulpragasam, 1986).

A leaf may have one or more points of infection and when several blisters developed on a single leaf, it becomes curled & distorted. The disease is more prevalent at the higher elevations with high relative humidity. Therefore it occurs mainly during the rainy seasons.

Experiments have revealed that a blister spore is relatively short lived (Loos and Portsmouth, 1949). Under dry but shady conditions spore remain viable for about a week. However, spores exposed for one hour to direct sunlight or to a temperature of 95^0 F, fail to germinate. This probably accounts for the failure of blister blight to establish itself in the low country.

This fungal is not known to attack any plant except tea (Petch, 1928). It was formerly suggested that blister blight of tea was identical with similar disease of Rhododendrons (Petch, 1928).

Until the advent of blister blight in 1946 there was no leaf disease of any economic importance in Sri Lanka. This is surprising in view of the humid conditions associated with tea culture, the large volume of leaf of all ages that is produced by the tea plant and the dense cover of foliage that is maintained. The other leaf diseases are caused by weak parasites and their attack is associated with prior damage to the leaf surface or general debility of the plant.

Concerning the controlling of blister blight in tea cultivation, Loss & Portsmouth describes the 3 main forms of control applications.

1. Protection of susceptible material with fungicides
2. Modification of existing agricultural practices.
3. Establishment of Blister blight resistant clones (Loos and Portsmouth, 1949).

At present it is controlled by regular application of copper fungicides because they are the cheapest and most effective fungicides available. In view of the rising cost of Cu and the ever-increasing wage structure, the cost of blister blight control has become major component in the cost of production. The Tea Research Institute has been regularly evaluating fungicides in order to find more effective and cheaper methods of control (Arulpragasam et al., 1987).

1.5. PHENOLS AND DISEASE RESISTANCE

A wide range of substances processing an aromatic ring bearing a OH substitute are called 'phenolic' substances. Phenolics show antifungal, antibacterial and antiviral activities. At high concentrations they inhibit spore germination and growth of fungi. Toxicity of phenolics varies depending upon their structure and in general, O-dihydroxy phenolics are highly toxic.

Some phenolics inhibit the production of fungal enzymes and inactivate the enzymes produced by pathogens. Phenolics may suppress toxin production by pathogens or detoxify the toxins produced by them (Vidyasekaran, 1988).

In some studies, total phenolic content in plant tissue have been correlated to disease resistance (Vidyasekaran, 1988). O-dihydroxy phenolics content has also been reported to be responsible for disease resistance and specific phenolics such as catechol, protocatechuic acid, chlorogenic acid, umbelliferone, scopoletin, catechin, gallocatechin, isoquerecetin ,anthocyanins have been observed to be involved in some specific resistant interactions (Vidyasekaran, 1988). It has also been reported the occurrence of pre-infectional phenolic inhibitors (Vidyasekaran, 1988). The changes of phenolics after infection have been corelated to disease resistance (Vidyasekaran, 1988: Punyasiri, et al., 2003).

Oxidized phenolics are more toxic than the un-oxidized phenolics and the oxidation is carried out by polyphenol oxidase or peroxidase. Hence, increased activity of these enzymes has been shown to be related to disease resistance. Spraying of ethylene or gibberellic acid or sugars, or application of K has increased the synthesis of phenolics and induced resistance (Vidyasekaran, 1988).

Tea Research Institute has developed blister blight resistant cultivars through conventional breeding programmes. However it takes long time to identify the blister blight resistant and susceptible cultivars through the conventional plant breeding programmes.

Identification of suitable chemical marker to detect blister blight resistant cultivars at the early stages of breeding programme would certainly help the tea industry, to manage the blister blight in an environmentally friendly manner.

1.6 OBJECTIVES OF THE STUDY

1. Determination of differences in chemical compounds in *Exobasidium vexans* resistant and susceptible cultivars in phase I and Phase II of the plant breeding programme.

2. Establishment of most suitable chemical compound which can be used as a chemical marker to detect resistant & susceptible tea cultivars at the early stages of breeding programme.

CHAPTER 02

LITRETURE REVIEW

2.1 CHEMISTRY & BIOCHEMISTRY OF TEA

The apical shoots of tea, consisting ideally of the terminal bud and two adjacent leaves referred as 'flush' make up the raw material which is processed to black, green, Oolong or other forms of tea. Flush has been studied by several workers with particular emphasis being paid to the group of polyphenols and their transformation products which are responsible for the unique character of tea (Bhatia and Ullah, 1968).

Table 2.1: Chemical components of fresh tea leaves (% on dry weight basis)

Water content:	75 – 78 %
Dry matters:	22 – 25 %
Organic Compounds:	
Proteins	(20 – 30 %)
Free Amino Acids	(1 – 4 %)
Alkaloids	(3 – 5 %)
Enzymes	
Tea polyphenols	(20 – 35 %)
Sugars	(20 – 25 %)
Organic Acids	(< 3 %)
Fat	(< 8 %)
Pigments	(< 1 %)
Aroma substances	(0.05 – 0.1 %)
Vitamins	(0.6 – 1.0 %)
Mineral Compounds:	
Water soluble	(2 – 4 %)
Water insoluble	(1.5 – 3 %)

Source: (Teranishi and Hornstein, 1995)

A tea drinker typically consumes 180 to 240mg of polyphenols from a strong cup of tea (Harbowy and Balentine, 1997). Recent interest in the health aspects associated with consumption of tea beverage has grown within the scientific community and has generated much excitement about tea polyphenols.

2.1.1 Polyphenols

The most important & characteristic components of tea leaves are the polyphenolic compounds; they are mainly responsible for the unique characteristic of processed tea (Roberts, 1962). Polyphenolic fraction represents 30 to 40% (w/w) of extracted solids (Harbowy and Balentine, 1997). They are responsible for most of the health aspects and antioxidative properties.

The term 'polyphenol' is an inclusive descriptor referring to the millions of natural and synthetic aromatic molecules that are substituted with multiple hydroxyl groups. The polyphenols comprise one of the most distinguish characteristics of the tea plant and have been more thoroughly investigated than any other class of compounds in tea (Harbowy and Balentine, 1997).

The polyphenols principally responsible for the colour, astringency and partially responsible for the flavour of the tea beverage. The compounds are known antioxidants and are being studied as an agents that might reduce risk factors associated with cancer and heart disease.

The polyphenols in tea can be subdivided into several chemical structures. Simple tea polyphenols are those that are synthesized during the early stages of polyphenols biosynthesis, whereas the degree of complexity of the polyphenols increased as one progresses down the biosynthetic pathway.

The flavonoids, a subgroup of polyphenols are the dominant class of green tea polyphenols that are synthesized part from the simple polyphenols and it represents compounds with 15 or grater carbon atom in the basic framework. The unique black tea polyphenols are the polyphenols of black tea; represent further chemical transformations of the green tea polyphenols (Harbowy and Balentine, 1997).

Basically polyphenols on fresh tea flush can be simply divided into flavanols, flavonols and flavanol glycosides, flavones and acids with depsides.

Out of those some 19 flavones have been detected in green tea (Harbowy & Balentine, 1997). Four of these were identified as vitexin, isovitexin, theiferin & theiferin B. Gallic acid is the predominant phenolic acid which can be found in tea.

And also theogallin and 3-galloylquinic acid is very important of its high level correlation to the quality of tea, and also because of its' high unique occurrence in tea (Teranishi and Hornstein, 1995). The mechanism of biosynthesis of polyphenols occurs by the well-established phenylalanine–shikimic acid pathway. However, there is some evidence for an alternative pathway which does not include phenylalanine as an obligatory intermediate (Teranishi and Hornstein, 1995).

2.1.1.1 Flavanols

Out of the polyphenolic compounds identified in fresh tea flush, flavanols are the major group of compounds present in tea (Harbowy and Balentine, 1997). Three sub grouping of the flavanols are afzelechin, catechin and gallocatechin. They represent the varying degree of benzene ring hydroxylation and they are the dominant forms, of which, the *epi*-isomers of the catechins and gallocatechins are the principle components found in tea. The tea catechins a term, commonly used to refer to both catechins and gallocatechins make up as much as 30% wt/wt of dissolved solids. A large percentage of the catechins present in tea exist as gallic acid esters while gallation is found to occur at the 3rd position of the carbon atom as shown in the Figure 2.2 (Harbowy and Balentine,1997). Tea Catechins have been widely used in the fields of medicine, daily necessities industry, and food and oil industry as ingredients of health foods, natural cosmetics and antioxidant of oil containing foods.

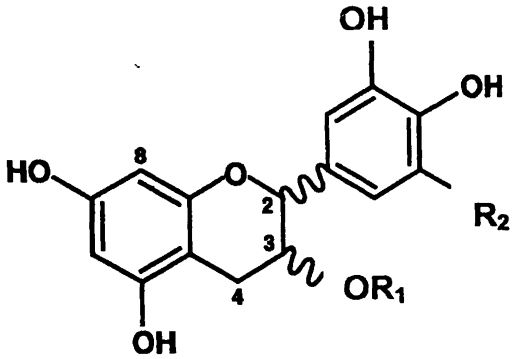

Figure 2.1: The principle tea catechins

		R1	R2
Epicatechin	EC	H	H
Epicatechin gallate	ECG	Gallate	H
Epigallocatechin	EGC	H	OH
Epigallocatechin Gallate	EGCG	Gallate	OH

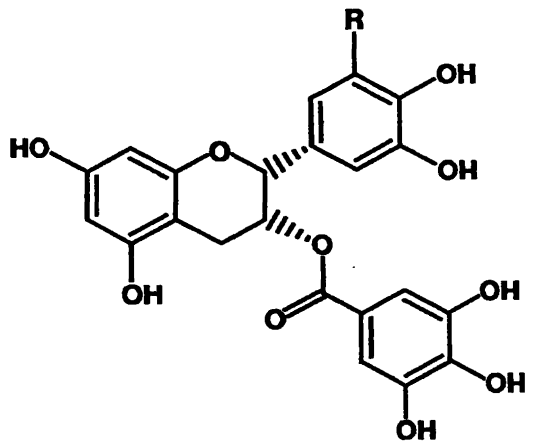

Figure 2.2: Chemical structure of Epicatechin -3-O-gallate

Monomers are exceedingly common in products like tea (catechin, gallocatechin, epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate) and cocoa (epicatechin). Epicatechin - 3 - O - gallate is a significant constituent of tea.

2.1.1.2 Flavonols

The flavonols (kaempferol, quercitin and myricitin) & their glycosides have also been recognized as an important components presents in tea. Majority of flavonols present in the initial fresh green leaf remain un-oxidized. Although there are some evidence that some of the flavonols are oxidized during fermentation (Harbowy and Balentine, 1997).

Table 2.2: Phenolic compounds in fresh tea flush

Compound	Amount in flush (% dry wt)
Flavanols	
(-) Epicatechin ; EC	1 – 3
(-) Epicatechin gallate; ECG	3 – 6
(-) Epigallocatechin; EGC	3 – 6
(-) Epigallocatechin gallate; EGCG	9 – 13
(+)Catechin ; C	1 – 2
(+)Galloatechin; GC	3 – 4
Flavonols & Flavonol Glycosides	-
Qurectin	-
Kaemperol	-
Qurectin-3-rhamnoglycoside	-
Kaemperol-3- rhamnoglysoside	-
Myricetin-3-glysoside	-
Flavons.	-
Vitexin	-
6,8-di-c-Glucosyl apigenin	-
Leucoanthocyanins	2 – 3
Acids & Depsides	2 – 3
Gallic acids	-
Chlorogenic acids(4 isomers)	-
p-Coumaryquinic acid	-
Theogallin	-
Ellagic acid	-
Total Polyphenols	25 – 35

Source: (Teranishi and Hornstain, 1995)

The polyphenolic composition in the tea leaf changes with leaf age and young leaves contain the highest amount of polyphenols. Phenolic patterns in different parts of tea plant vary according to their morphological affinities to the leaf (Bhatia and Ullah, 1968).

The site of biosynthesis of polyphenols in tea leaf is unknown but polyphenols are localized in the vacuoles of the palisade cells of the leaves (Selveadran and King, 1976). The enzyme Polyphenol oxidase are located in the outer or epidermal layer of the leaf.

During the processing of black tea, about 90-95% of the flavanols undergo enzymatic oxidation to products which are directly responsible for the characteristic colour, astringency and unique taste of the tea brew.

2.1.2 Enzymes

Polyphenol oxidase (PPO) is one of the important enzymes involved in the formation of black tea polyphenols. This enzyme is a metello protein, thought to contain a binuclear copper active site. PPO is localized within plant cells in the mitochondria, the chloroplasts and the peroxisomes. PPO becomes activated when plant tissue is damaged catalyzing the formation of phenolic polymers. Most of PPO enzymes have a pH optimum in the rage of 5.0 to 7.0.

2.1.3 Amino Acids

The amino acids present in tea have been identified as aspartic acid, threonine glutamic acid, glycine, alpha alanine, valine, methionine, isoleusine, leucine, tyrosine, phenylananine, lysine, histidine, argentine, glutamine, asparagines, tryphtophan and theanine (Bhatia and Deb, 1965). Theanine is the most abundant and accounts for 50% of the total amino acids and 1% of the dry weight of tea.

2.1.4 Caffeine

Caffeine content of tea ranges from 2.5 % to 4.0 % and varies according to the season, plucking standard and the cultivar. The mild stimulant effect of tea is due to the presence of caffeine and it makes a significant contribution to the briskness and creaming properties of tea (Hara et al., 1995). The tender young leaves of tea bushes contain higher concentration of caffeine than older leaves, with the highest concentrations in the terminal bud.

2.1.5 Chlorophyll

Chlorophyll content varies with the cultivar as well as with climatic conditions (Teranishi and Homstein, 1995). Chlorophyll present in fresh tea leaves are converted to phaeophytin and phaeophorbide during processing. These compounds play an important role in determining the color of made tea.

There is a large decrease in chlorophylls during the processing of black tea, and this decrease can be accounted for their transformation of phaeophytins and phaeophorbide. It will determine whither the appearance of the tea will be black (phaeophytins) or brown (phaeophorbide). The mechanism of degradation of chlorophyll during processing is shown in figure.

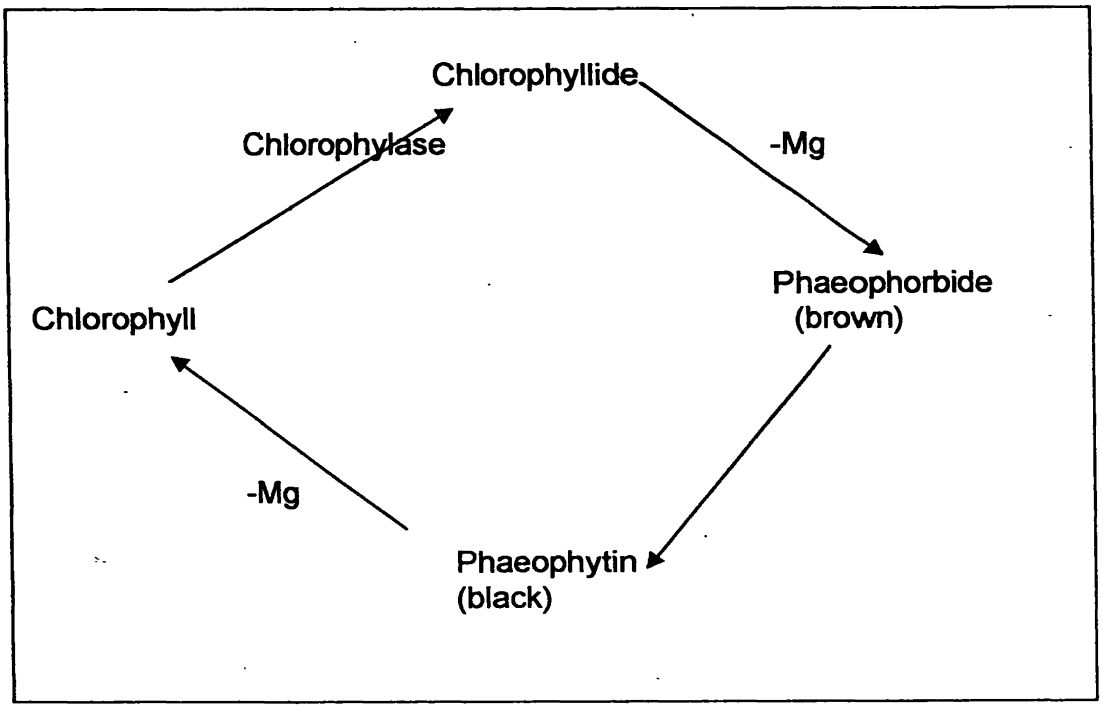

Figure 2.3: Changes undergone chlorophyll during processing of black tea.

2.2 TEA PLANT & BLISTER BLIGHT LEAF DISEASE

2.2.1 History of blister blight

Blister blight leaf disease was first mentioned in India about the year 1868. In 1903 Watt and Mann, two advisory officers worked un the India Tea association in Assam were reported that "so far as we have been able to observe this blight is, an exclusively confined to upper Assam. It has never been definitely reported below the Jorhat district in the South, and the North Lakhimpur districts in the North, bank. It has never visited either Cachar, Sylhet, Darjeeling or the Duars" (Petch, 1928).

For about 40 years, blister blight was known only in upper Assam. However in 1908, severe attacks broke out in the Darjeeling districts and since that date it has been permanently establish there.

Thus, the first appearance of the disease was in the extreme North-east of the tea districts of Northern India and 40 years later it appeared in the North-west corner of the northern India. After about 20 years it has been found in the extreme south. It may be said that blister blight now occurs at the 3 corners of the triangle covered by the north Indian tea districts. It was formerly stated that blister blight would flourish only in the cooler tea districts, where the fungus may be found at any time of the year on bushes growing in damp shady places (Petch, 1928).

Exobasidium vexans is not known attack any plant except tea. Mr. A.C. Tunstall (former mycologist of the scientific department of the Indian Tea Association) states that an *Exobasidium vexans* occurs on *camellia drupifera* in the jungle of the north of the Surma valley, but it is a different species (Petch, 1928).

2.2.2 Occurrence of blister blight leaf disease in Sri Lanka

In October ,1946 blister blight was reported from an estate in the Dolosbage district of Ceylon. This incidence of the disease followed close on its observance in the tea districts of south India. In short space of time the attack spread through the tea areas which receive the south west monsoon. At the end of the 1946, areas subject to the north east rains, received their first visitation of the fungus. Since then probably every tea estates has had its' attacks which may occur as light or heavy infestation dependant on climatic or environmental conditions. Much has since been written and said both of the disease and its control. When the disease first became general in our tea areas, instructions on control measures had to be based on the evidence collected from other countries where it had been known for many years (Portsmouth and Loos, 1949).

Elevation plays an important part in the severity of the disease. Blister blight is of no economic importance below 1000 feet and extensive control measures on estates below this elevation are therefore unnecessary. At higher elevations severity of attack depends mainly on climatic conditions.

2.2.3 Causative organism

The pathogen, *Exobasidium vexans* belongs to the family Exobasidiaceae of order Exobasidiales. It is an obligate parasite and so far, there is no response of its growth in axenic culture (Tubbs, 1947).

2.2.4 Specific characteristics of the disease

The most important and fortunate characteristic of the disease is that it can infect only young and succulent leaves and stems and does not affect the mature leaves. As the leaves become older they develop resistance to infection (Arulpragasam, 1992).

2.2.5 Life cycle of blister blight

The disease is spread by windborne basidiospores. Under suitable conditions a spore coming into contact with the upper surface of a young leaf or stem, germinates within 6-10 hours and forms an appressorium (or infection structure). During this period the fungus is very susceptible to desiccation and the fungicidal effect of sunshine and chemicals. Between 10-14 hours after the formation of the appressorium the fungus penetrates the leaf surface. Now it is protected from external agencies like sunshine and chemical spray deposits on the leaf surface.

Previous work carried out in other countries suggested that entry of the fungus into a leaf was through the stomata (breathing pores) which are located on the under surface of the leaf (Arulpragasam, 1986).

However later, research work has proved that entry of the fungus is mainly through the leaf cuticle on either the upper or lower surface of the leaf and that there is no stomatal attraction (Portsmouth and Loos, 1949). The fungus utilizes the leaf tissue for its growth. 8-10 days after penetration the first visible sign of infection appears as a translucent spot which is a result of the disorganization of the leaf tissue.

The fungus ruptures the lower epidermis of the leaf and begins to produce basidiospores 15-17 days after penetration. A mature sporulating blister therefore is produced 18-21 days after penetration. The spores are liberated in enormous numbers from each blister into the atmosphere. A blister may sporulate for 7-8 days before it begins to die. This is indicated by the browning of the blistered area (Arulpragasam, 1986).

2.2.6 Symptoms of the disease

2.2.6.1 Leaf symptoms

The first visible symptom of the disease is a pale yellow or lime green spot which is more translucent than the rest of the leaf. The spot may sometimes be pink red or brown depending on the reaction of the different tea cultivars to the infection and the spot gradually enlarges in the form of circles of increasing diameter up to a maximum size of about 12.5mm. The rate of enlargement of the blister depends on environmental conditions as well as an internal physiological characteristic of the leaf. As the disease progresses inside the leaf the upper surface of the developing blister becomes indented corresponding with a protrusion of the lower surface which becomes white and powdery as spore production begins. After sporulation the blister begins to dry up starting at the centre. The blistered area becomes brown as the leaf tissues die. A leaf may have one or more points of infection when several blisters develop on a single leaf, it becomes curled and distorted.

2.2.6.2 Stem symptoms

When the fungus infects young stems the damage to the plant is more serious than when leaves are attacked. This is because the infected stem often breaks off and dies back resulting in set back to growth. The mature blisters appear as white velvety patches of irregular shape as oppose to the circular ones of the leaves. Infected patches are usually thicker than healthy areas. Stems are often bent and distorted and may break off and die back at the point of infection. Repeated stem infections can weaken the plants considerably and if allowed to go on without proper control measures being adopted the plants will eventually die.

2.2.7 Distribution and time of occurrence of the disease

All tea estates above 600m receiving the south-west and north east monsoon rains and areas in the Uva region are affected during the north east monsoon. The disease is more prevalent and severe at higher elevations.

2.2.8 Climatic conditions favouring the disease

Moisture is the most essential factor required for the infection of tea by the fungus. Moisture is necessary for the germination of the spore and high relative humidity is required for spore production and spore release. Mist, cloud, and rain increase the incidence of blister blight, whereas desiccation and exposure to direct sunlight kills the spores of the fungus.

2.2.9 Control of blister blight in tea lands

As being the blister blight leaf disease, a major economically important disease in tea industry, it is important to identify the controlling of blister blight leaf disease through the tea cultivation.

Presently controlling of blister blight comes under two methods.

1. Use of resistant cultivars
2. Chemical control

1. Use of resistant cultivar

Growing resistant cultivars is the best option to control the blister blight. So far no clones have been found to totally resistant t this disease. Only gradation of susceptibility has been found in the different type of tea cultivars (Anon., 2002). Usage of resistant cultivars will reduce the amount of money spent for chemical control methods.

2. Chemical control

Chemical control can be done by using two fungicides

1. Protective fungicide- Copper fungicide
2. Curative fungicide- Systematic fungicide

1. Copper fungicide

The main ingredient of these fungicides is metallic copper. This type of fungicides gives adequate protection against blister blight in tea. When sprayed copper, deposited on the leaf surface & prevents the germination of spores, there by preventing infection.

However, copper will have no effect if applied after the fungus has penetrated the leaf surface. According to the solubility, the copper fungicides have been categorized into two major group (Anon., 2002). They are;

Group (a)-Copper Oxide & copper Oxychloride (insoluble in water)

Group (b)-Copper hydroxide (slightly soluble in water)

2. Systematic fungicides

This type of fungicides give better control of disease & it has both protective and curative properties. Unlike the protective fungicides, they can cure an established infection. The chemical composition of the presently recommended fungicides fall into two broad category (Anon., 2002). They are Triazoles (e.g. Baycor, Folicur, Tilt, Bumpre) and Morpholine derivative (e.g. Calixin).

Presently both these fungicides are being used extensively in most tea growing area. The main disadvantage of the continuous use of fungicide is the fungus developing the resistance to the disease. Other than that, the high costs and potential residues in made tea also the disadvantages of the continuous use of fungicides.
This can be minimized by adopting the following strategies.

1. Using the lowest possible concentration of fungicides as recommended by the TRI.
2. Alternating the use of systematic fungicides with protective copper fungicides.
3. Using fungicides that belong to different groups, possessing different modes of action.

2.3 PATHOLOGICAL FUNCTION OF PHENOLIC COMPOUNDS IN PLANTS

Higher plant must protect themselves from invasion by microorganisms through physical barriers and the production of a wide range of secondary chemicals. Many of these compounds also confer selective advantages by inhibiting competing plants. The plant phenolics constitute the largest group of plant secondary compounds, primarily synthesized through the shikimic acid pathway. They constitute an extremely diverse group, many of which have been shown to be antimicrobial and phytotoxic. Flavonoids are a biologically important and chemically diverse group that can be further divided into subgroups including anthocyaidins, flavones, flavanones, flavonols, flavanols, isoflavones & chromones (Berhow and Vaughn, 1999).

From the early investigations it has been found that there is an association between the presence of toxic compounds and the extent of growth of fungi in plant tissues (Harbone, 1964). In discussing the relationships between the pathogen *Puccinia dispersa* and the resistance to infection of certain species of *Bromus,* Marshall Ward (1905) emphasized that "infection and resistance to infection depend on the power of the fungus protoplasm to overcome the resistance of the cells of the host by means of enzyme or toxin; and reciprocally, on that of the cells of the host from antibodies

which destroy such enzymes or toxins or to excrete chemotactic substances which repeal or attract the fungus protoplasm".

He concluded that the factors inhibitory to the pathogen must be more than a mere soluble poison oozing from the host cells. It is thus clear that ward recognized the significance of fungus being inhibited after penetration of its' host and the dynamic nature of disease resistance.

In 1909 Bernard described the immunity in plants from the point of view of the infection of orchid tubers by mycorrhizal fungi (Harbone, 1964). One of his paper he described his diffusion technique in which he placed live and dead fragments of Orchid tuber on agar and inoculated the agar surface with *Rhizocotonia repens* of a short distance from them. He observed inhibition of growth of the fungus in the vicinity of the live, but not of the dead, fragments of tuber tissues; thus he reasoned that a metabolite diffused from the fungal culture to the Orchid tissues and that an interaction occurred between the fungal metabolite and the components of the orchid tissues.

He concluded that as a result of the host parasite interaction, a second substance was produced which diffused back to the fungus and inhibited its subsequent growth (Harbone, 1964).

After the Ward & Bernard investigations, the concept that toxins are formed either before or after infection has been examined in relation to disease resistance by many worker and several modification of the theory have appeared.

The clear example of a flavonoids being implicated in disease resistance in plants is the Pisatin, the phytoalexin of *Pisaum sativum* discovered by Cruickshank & Perrin in 1960. Pisatin was isolated from pea pods infected with *Monolinia fructicola* and identified as the isoflavonoid by Perrin and Bottomely in1962 (Harbone, 1967). The role of pisatin in disease resistance of pea plants, has been discussed by Cruickshank & Perrin. Pisatin is absent in healthy plant tissues but it is produced in root, leaf, stem and any type of fungus. It's significant, that if produced in sufficient concentration , it is toxic to an invading fungus and prevents its multiplication (Harbone, 1967).

Among the huge group of flavonoids, the monomeric flavan-3-ols together with their oligomeric derivatives, the proanthocyanidins, possess some special properties that give them a great significance in plant defense (Feucht and Treutter, 1999). The function of flavan-3-ols in plant physiology range from scavenging of free radicals to growth regulating activity from protein precipitation to DNA protection, and from hydration to impregnation of macromolecules of the cell wall (Feucht and Treutter, 1999).

Taking of the antibacterial effects of the flavan-3-ols, *Staphylococcus aureus* ,*Lactobacillus casei* & *Eschrechia coli* is inhibited in the presence of procyanidins and prodelphinidins isolated from grapes (Feucht and Treutter, 1999). Epicatechin-3-O-gallate and the Epigallocatechin-3-O-gallate affect the growth of *Streptococcus mutants* and catechin & epicatecin were reported to impair *Pseudomonas Maltophilia* and *Enterobacter cloacae* (Feucht and Treutter, 1999). The antifungal activity of flavan-3-ols are mentioned in the table.

Table 2.3: Fungitoxicity of flavan-3-ols

	Microorganism
1. Catechin	*Rhizoctonia solani*
	Trichoderma viride
	Fomes annosus
2. Procyanidin	*Rhizoctonia solani*
Fraction from *Pinus sylvestris*	
Fraction from *Anona squamosa*	
3. Propelargonidin	*Rhizoctonia solani*
Fraction from *cassia javanice*	
4. Fraction from *Peltophorum pterocarpum*	
5. Prorobinetinidin	
Fraction from *Acasia leucophloea*	

Source: (Feucht and Treutter, 1999).

It has been reported that the lesions produced by apple tissues following an attack by the fungus *Venturia inaequalis* are surrounded by some cell layers rich in flavan-3-ols. The accumulation of epicatechin and procyanidins is induced by the action of the fungus and may be attributed to an unspecific wound response, since the fungus produces pectinase and celluases (Feucht and Treutter, 1999) which destroy the cell wall.

As plant developed the use of flavan-3-ols for defense, several organisms adapted to these compounds and developed their own defensive systems. It was found that some *Rhizobium* & *Fusarium* species are able to cleave the aromatic ring (Feucht and Treutter, 1999). Some species of the *Colletotricum* produce special protein that inactivate the phenols by binding them, and thus increasing the virulence of the pathogen.

According to the unifying concept of flavanols & defense (Feucht and Treutter, 1999) the integrated defense processed against biotic or abiotic stress included that the dimeric, trimeric and tetrameric proanthocyanidins (flavanols) were found to be prominent protein kinase inhibitors mediating defense function related to herbivory and fungal invasion (Feucht and Treutter, 1999).

It is evident that flavanoids play a major role in plants. Although much basic research remains to be carried out, it is possible that many of these compounds, either as isolates or in conjunction with other compounds may be used in either agricultural or pharmaceutical roles. The use of flavanoids and other plant phenolics as biopesticids either as a applied pesticide or through genetically engineered plants, has been widely discussed (Berhow and Vaughn, 1999).

From the previous studies conducted by Greathouse and Rigler in 1940 (Harbone, 1964), have tested 45 phenolics and related compounds. They found that the introduction of second hydroxyl group into phenol decreases toxicity, while a similar introduction into benzoic acid increases toxicity. Similarly, substitution of phenol in the *ortho* position with a basic amino group increase toxicity. The same group introduced in to the *meta* or *para* position in the ring reduces toxicity.

CHAPTER 03

3. METHODOLOGY

3.1 EXPERIMENTATION

3.1.1 Location

The experiment was carried out at field No 12 of the St. Coombs estate, Tea Research Institute, Thalawakelle.

3.1.2 Selection of cultivars

Seven accessions from phase I (phase 1- VP 82) and nine accessions from phase II (phase II -VP 81) of the conventional breeding programme were selected for the study. These accessions were categorized to blister blight resistant and susceptible as for the observation make by the Pathology division, Tea Research Institute.

Phase I - VP 82		*Phase I I- VP 81*	
Resistant	Susceptible	Resistant	Susceptible
19	Y8	58	61
27	Y6B	118	39
31	Y6	48	2
	Y5	119	24
		43	

DT1 (Blister blight resistant) & TRI 2024 (Blister blight susceptible) were use as the standards.

3.1.3 Sampling

Tender leaves (normally two leaves & bud) of accessions were collected from each accessions for chemical analysis. The samples were replicated 3 times.

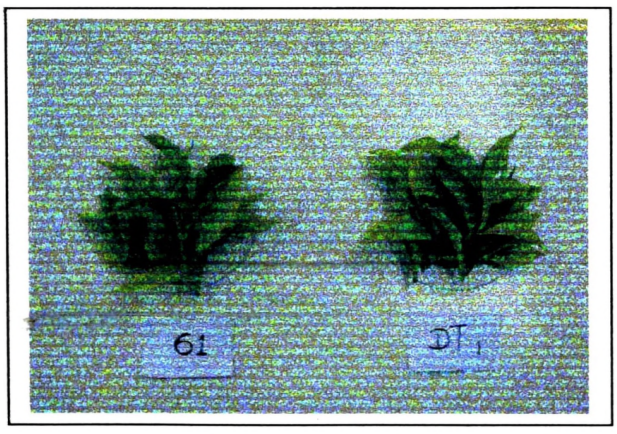

Figure 3.1: Two leaves & a bud

3.2 ANALYSIS OF FLUSH

3.2.1 Moisture determination

5g of the sample was weighed and dried at 104^0C for 6 hours. The dry matter content was measured after 6 hours.

3.2.2 Sample preparation-(Extraction of samples for the analysis of individual catechins)

50ml of boiled (70%v/v) methanol was added to 10g of flush and kept for 10 minutes. The flush was cooled and macerated in a homogenizer (Ultra-turrax macerator) for 5 minutes. Resulted solution centrifuged at 4000 rpm for 5 minutes.

The supernant was transferred to a 100ml volumetric flask and the remain was re-macerated with 50ml, 70%(v/v) methanol. Finally it was centrifuged at 4000rpm for about 5 minutes. Supernant was transferred into the same 100ml volumetric flask and the volume was made up to 100ml with 70%(v/v) methanol. The prepared samples were thoroughly shaken and filtered through 0.45µm filters.

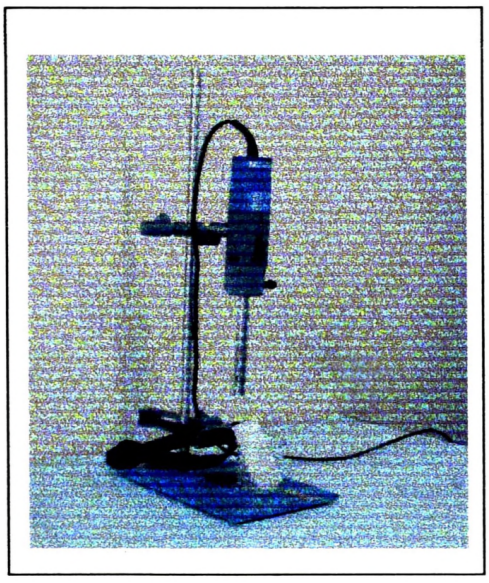

Figure 3.2: Ultra-turrax macerator

3.2.3 Determination of Individual catechins

3.2.3.1 Reagents and Apparatus

Reagents

1. Water, conforming to grade 1 of ISO 3696:1987
2. Acetonitril, HPLC grade
3. Acetic acid
4. Methanol
5. Methanol/water extraction mixture, 70% v/v Methanol
6. EDTA solution
7. Caffeine standard solutions A, B, C

Apparatus

1. High Performance Liquid Chromatography system
2. Membrane filters of 0.45µm pore size

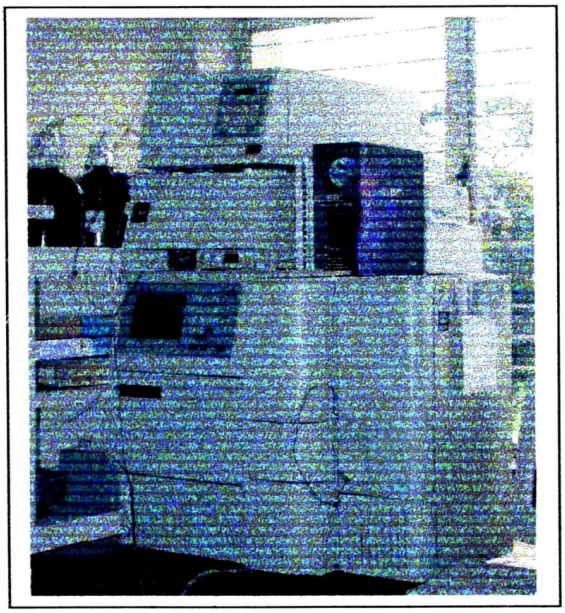

Figure 3.3: HPLC machine

3.2.3.2 HPLC mobile phases

Preparation of Mobile phase A (9% v/v acetonitril, 2% v/v acetic acid, 20µg/ml EDTA)

180ml of acetonitril and 40ml of acetic acid were transferred in to a 2 liter volumetric flask. Approximately 100ml of water and 2.0ml of EDTA solution were added. It was diluted to the mark with water, mixed and filtered through 0.45µm pore size.

Parasitic infection induces metabolic dysfunction of the host plant cells at the site of infection. It may involve cells adjacent to the site of infection but it is essentially a localized interaction between the potentially parasitic fungus and the host cells being invaded. Dysfunction occurs independently of the final response of the host tissue and is expressed in terms of increased respiration increased protein synthesis and increases polyphenol accumulation. Most of these changes do not appear to be directly concerned with disease reaction. But are merely results of infection.

Preparation of Mobile phase B (80% v/v acetonitril, 2% v/v acetic acid, 20µg/ml EDTA)

800ml of acetonitril and 20ml of acetic acid were transferred into one-mark volumetric flask. Approximately 100ml of water and 2.0ml of EDTA solution were added and diluted to the mark with water, mixed and filtered through a filter 0.45µm pore size.

Preparation of mixed standard solutions A,B & C

Three mixed standard solution A, B & C was prepared containing caffeine. Concentrations of the Caffeine solutions were 50µg/ml, 100µg/ml and 150µg/ml respectively (Standards were prepared according to the Document ISO/TC 34/SC 8).

3.2.3.3 HPLC analysis

The sample was filtered through 0.45µm filter and 10µl of this was injected to the HPLC system (Waters Alliance 2690XE Separation module coupled to a Waterd 996 photodiode array detector (PDA) and Waters Millenium 32 data system). The Column is Phenomenex-phenyl hexyl Column (4.6*250mm) and, a linear gradient of mobile phase A, and B was used for the separation.

3.2.3.3.1 Chromatographic conditions

1. Flow rate of the mobile phase is 1.00ml/min.
2. Binary gradient condition

   100% mobile phase A for 10 min, then over 15 min a linear gradient to 68% mobile phase B and hold at this composition for 10 min. Then reset to 100% mobile phase A & allow to equilibrate for 10 min-before next injection.
3. Temperature of the column 35^0C.
4. UV detector set at 278nm.

Once the flow rate of the mobile phases and temperature are stable, 10µl of each of the mixed standard solution A, B & C was injected. This was follows by the injection of 10µl of the sample.

The mixed working standard solution was injected at regular intervals. Data was calibrate using Millenium 32 data system.

3.3 Calculation

Quantitation using a caffeine standard and catechin Relative Response Factors (RRFs)

Using given three caffeine standard solutions, a linear calibration graph was constructed against caffeine peak areas obtained for each the A, B & C standard solution. Finally, slope & intercept values were obtained.

Individual catechins content can be calculated as a percentage by mass on a sample dry matte basis;

Formula as follows;

$$= \frac{(A_{sample} - A_{intercept}) * RRF_{Std} * V * d * 100}{Slope_{Std} * m * 10000 * DM}$$

Where,

A_{sample}	is the peak area of the individual component in the test sample;
$A_{intercept}$	is the peak area at the point the caffeine calibration line intercepts the y-axis;
$Slope_{std}$	is the caffeine calibration line slope;
RRF_{std}	is the Relative Response Factor, measured with respect to caffeine for the individual component;(Table 3.3)
V	is the sample extraction volume;
d	is the dilution factor ;
m	is the mass, in grams of the sample test portion;
DM	is the dry matter content, expressed as a mass fraction in percent of the test sample

The results were statistically analyzed using Statistic Analysis System (SAS) and Minitab. Mean comparisons was done using Dunnet's test.

CHAPTER 04

4. RESULTS & DISCUSSION

4.1 CATECHINS & CAFFEINE CONENTS OF THE SELECTIONS FROM PHASE I

Results of caffeine & catechin analysis of fresh tea flush of the selection from phase I of the breeding programme are given in table 4.1

Table 4.1: Catechin and Caffeine content of selections from phase I

Resistant(R) or Susceptible(S) (Preliminary ratings given by the Pathology Division, TRI)	Selection/ Cultivar	mg/g				
		EGC	CF	EC	EGCG	ECG
R	**DT1**	31.82	37.73	17.42	80.12	37.52
R	19	▼45.86*	▼44.45	14.59	▼112.29*	▼30.85
R	27	42.02	42.92	12.48	105.53*	▼29.10*
R	31	31.60	36.37	16.39	81.43	36.32
S	Y8	24.24	35.14	16.88	101.23*	▼52.46*
S	Y6B	▼45.61*	37.87	14.37	81.59	▼22.70*
S	Y6	▼45.47*	34.87	16.79	82.07	▼27.21*
S	Y5	27.08	39.50	10.44*	▼121.55*	38.67
S	**2024**	23.21	35.56	11.96	89.27	40.23

▼ significantly different when compared to TRI 2024

* significantly different when compared to DT1

EC- Epicatechin
EGC- Epigallocatechin
EGCG-Epigallocatechin gallate
ECG-Epicatechin gallate
CF- Caffeine

The catechin and caffeine levels of the selections were compared with that of established TRI recommended cultivar TRI 2024 (Susceptible to blister blight) and DT1 (Resistant to blister blight). All the results were obtained using the peak areas of the graphs obtained from the High Performance Liquid Chromatography system. (Figure 4.1, Figure 4.2 & Figure 4.3)

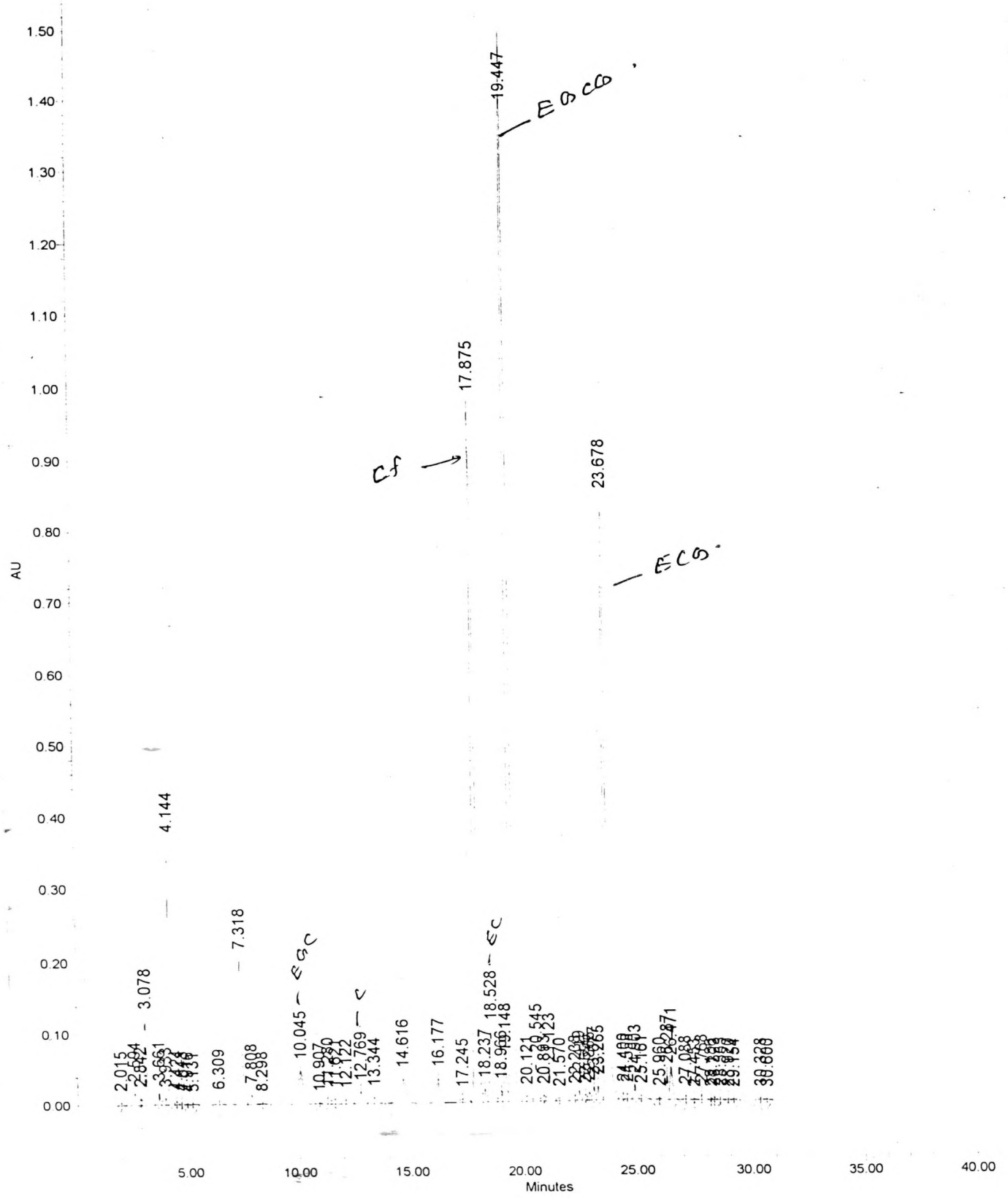

Figure 4.1: Peak areas obtained from the HPLC for TRI 2024

Figure 4.1: Peak areas obtained from the HPLC for TRI 2024

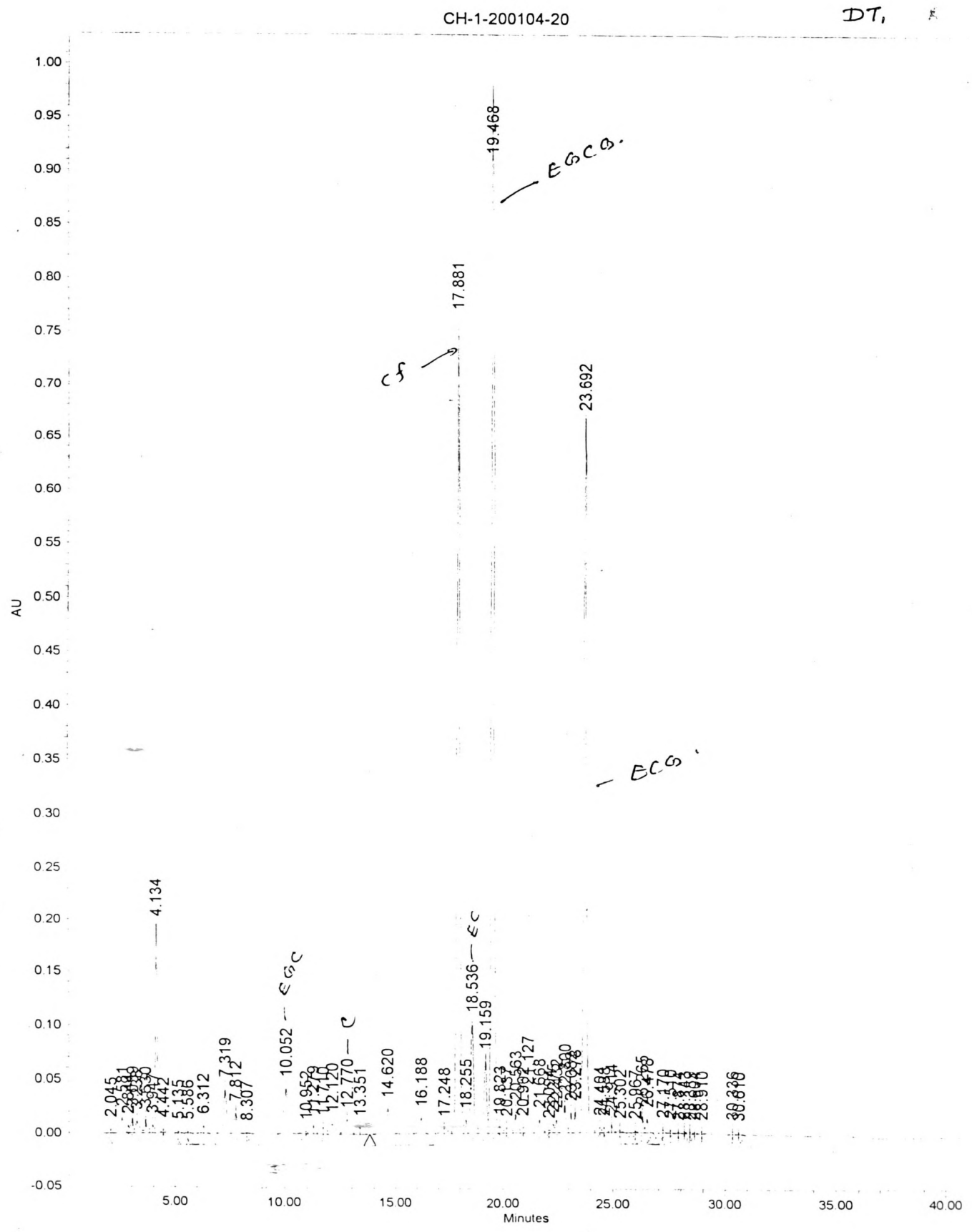

Figure 4.2: Peak areas obtained from the HPLC for DT1

Figure 4.2: Peak areas obtained from the HPLC for DT1

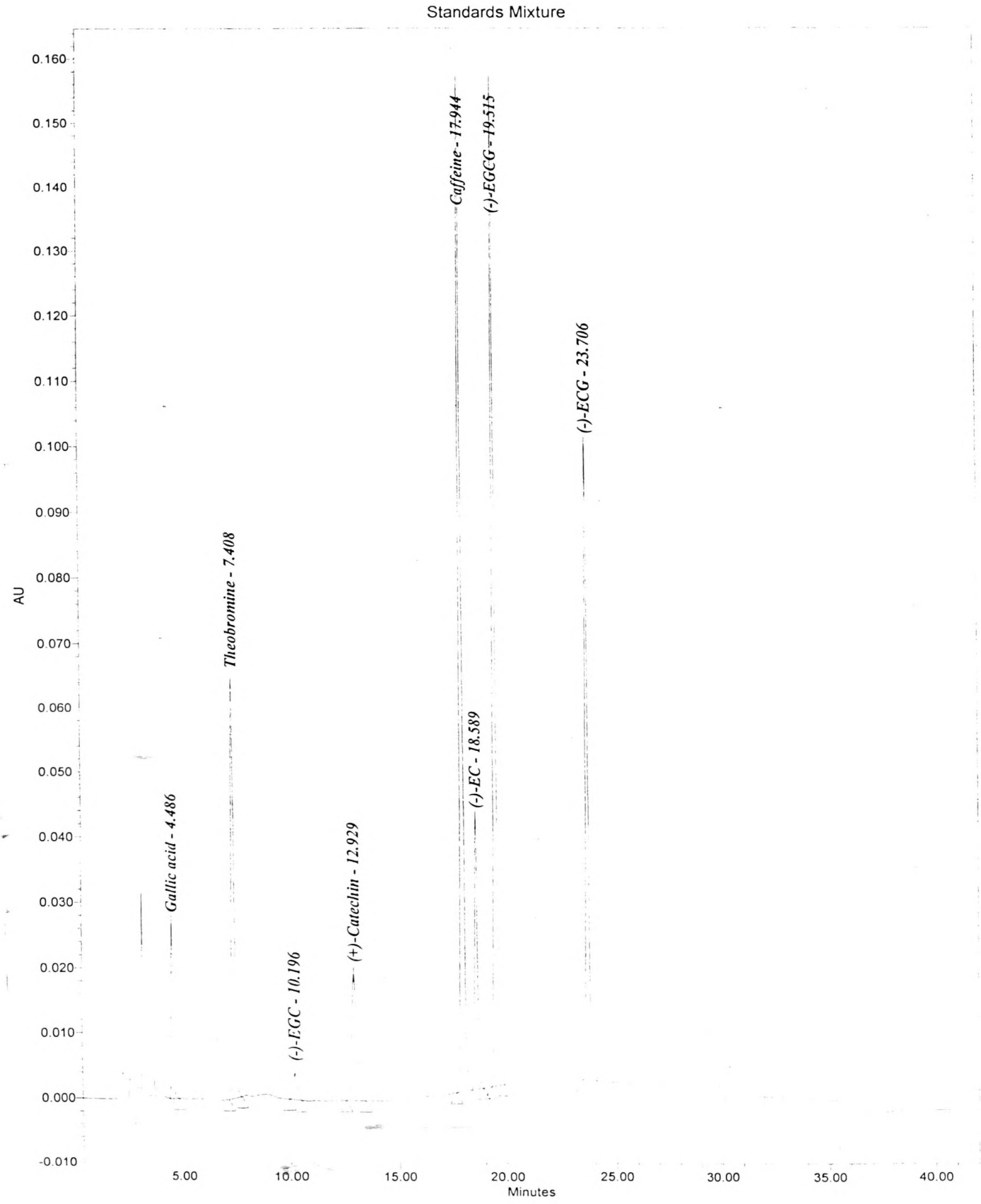

Figure 4.3: Peak areas obtained from the HPLC for standard mixture of polyphenols

Figure 4.3: Peak areas obtained from the HPLC for standard mixture of polyphenols

4.1.1 Selections from phase I- EGC content

Selection 19 (45.86mg/g), Y6B (45.61mg/g) and Y6 (45.47mg/g) showed significantly higher levels of EGC when compared with DT1 (31.82mg/g) and TRI 2024 (23.21mg/g) (Table 4.1). According to the preliminary observation made by Pathology division, selection 19 is a blister blight resistant and Y6B and Y6 are susceptible selections. Therefore the results indicates that there is no correlation between EGC content in the tea plant and blister blight resistance. Studies carried out by Punyasiri et al, (Punyasiri et al., 2003) also confirmed these observation.

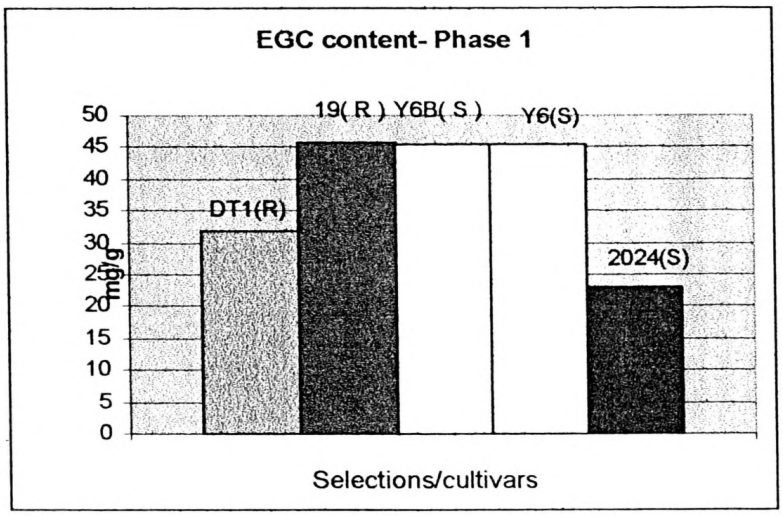

Figure 4.4: Comparison of EGC content in phase I selections

4.1.2 Selections from phase I- EC content

Selection 19 (14.59mg/g) gave significantly higher values for EC when compared with both TRI 2024 (11.96mg/g) and DT1(17.42mg/g)(Table 4.1). EGCG content of selection Y8 (101.23mg/g) was significantly higher than that of the resistant cultivar DT1. It is interesting to note that Y5 selection gave significantly low levels of EC and significantly high level for EGCG indicating that Y5 selection is highly susceptible to blister blight disease (Figure 4.5 & Figure 4.6). This is quite in agreement with the observation made by Plant Pathology division as well as preliminary work done by Punyasiri et al, (Punyasiri et al., 2003). These selections has to be monitored over a period of time for catechin content before making any conclusion whether we could use these chemical parameters to select blister blight resistant verities at an early stages of breeding programme.

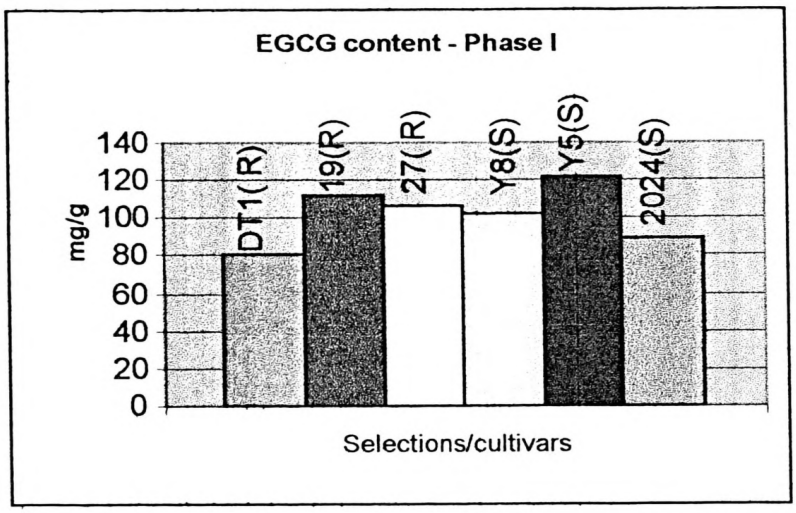

Figure 4.5: Comparison of EGCG content in Phase I selections

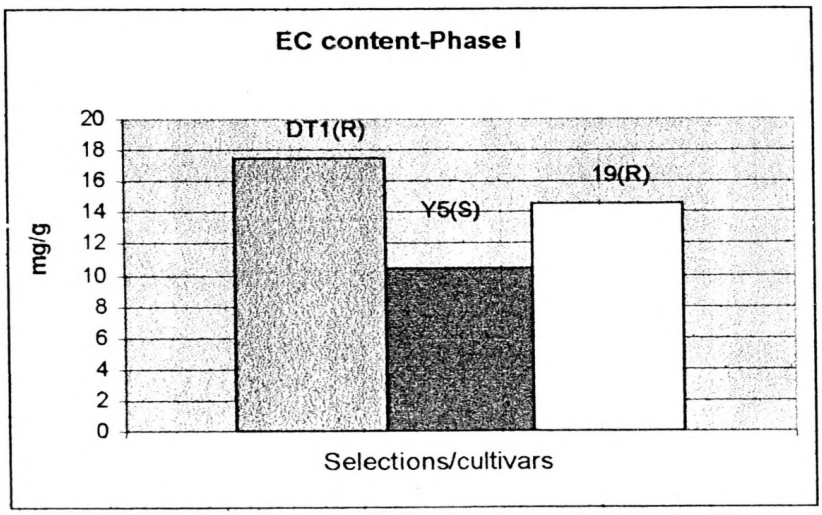

Figure 4.6: Comparison of EC content in phase I selections

4.1.3 Selections from phase I- ECG content

Selection 27, Y6B and Y6 showed significantly lower levels for ECG when compared with both DT1 and TRI 2024(Table 4.1).

Selection Y8 showed significantly higher levels of ECG when compared with DT1 and TRI 2024. However the levels of ECG in the selection 19 is significantly lower than TRI 2024 (Figure 4.7). From these observations it is not possible to establish any correlation between the levels of ECG in the tea plant and blister blight resistance.

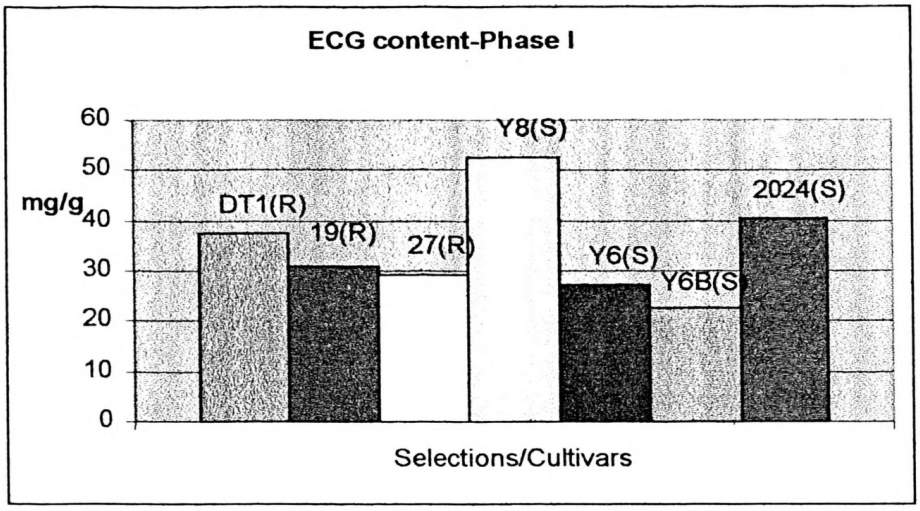

Figure 4.7: Comparision of ECG content in phase I selections

The observation made from phase I selections indicate, that it may be possible to use EC levels together with EGCG levels in order to detect blister blight resistance or susceptibility. However it is essential to monitor catechin and caffeine levels in these selections and compare these levels with the blister blight resistance ratings given by the Pathology division before we make any recommendations on the use of these chemical markers as a tool for the identification of blister blight resistant varieties in the breeding programme.

4.2 CATECHINS & CAFFEINE CONENTS OF THE SELECTIONS FROM PHASE II

Results of catechin and caffeine analysis of fresh tea flush from phase II of the breeding programme are given in table 4.2.

Table 4.2: Catechin and caffeine content of selections from phase II

Resistant(R) or Susceptible(S) (Preliminary ratings given by the Pathology Division, TRI)	Selection/ Cultivar	mg/g				
		EGC	CF	EC	EGCG	ECG
R	**DT1**	31.82	37.73	17.42	80.12	37.52
R	58	15.94*	33.72	12.61	81.58	41.01
R	118	26.62	26.69	14.11	▼55.58	▼23.52*
R	48	▼40.41	34.85	17.36	74.78	▼25.84
R	119	▼41.30	37.69	▼19.16	67.62	30.05
R	43	23.37	31.30	11.91	68.95	▼27.15
S	61	15.70*	15.70	11.25	85.55	45.53
S	139	24.40	24.40	8.49*	▼59.41	▼17.30*
S	2	29.31	35.62	17.96	81.88	39.93
S	24	36.05	30.51	16.17	72.09	29.25
S	**2024**	23.21	35.56	11.96	89.27	40.23

▼ significantly different when compared to TRI 2024

* significantly different when compared to DT1

4.2.1 Selections from phase II – EGC content

Selection 48(40.41mg/g) and 119(41.30mg/g) gave significantly higher values for EGC when compared with TRI 2024(23.21mg/g)(Table 4.2). Selections 61(15.70mg/g) gave a significantly lower value for EGC when compare with DT1(Figure 4.8). As in the case of phase I selections, EGC levels precent in the selections did not show any correlation to blister blight disease resistant.

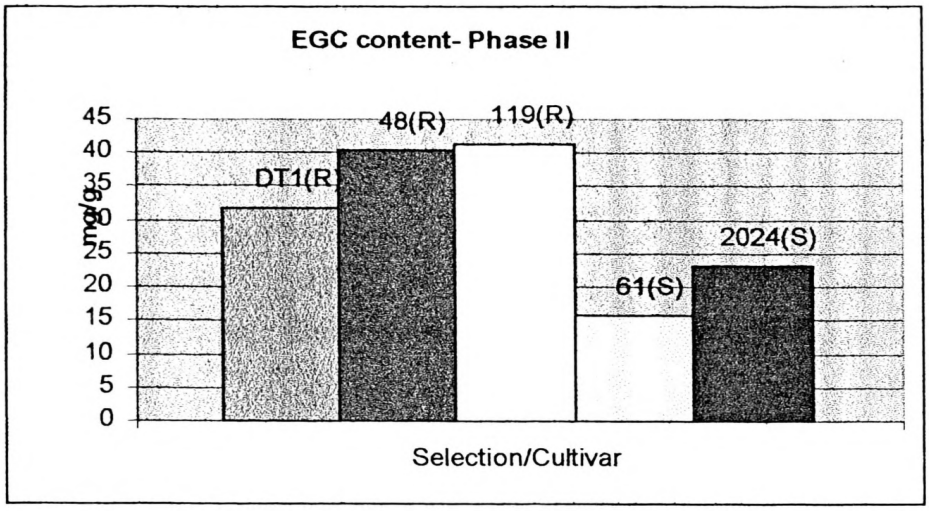

Figure 4.8: Comparison of EGC content in phase II selections, TRI 2024 & DT1

4.2.3 Selections from phase II- EC content

The selection 119 (19.16mg/g) resulted significantly higher value for EC when compared with TRI 2024(11.96mg/g)(Table 4.2). Selection 139(8.49mg/g) which is a susceptible selection, gave significantly lower levels of EC when compared with DT1(17.42mg/g)(Figure 4.9). These findings supports the earlier observation made by Punyasiri et al, (Punyasiri et al., 2003). Thereby indicating a possible correlation between EC content present in the tea plant and blister blight resistance.

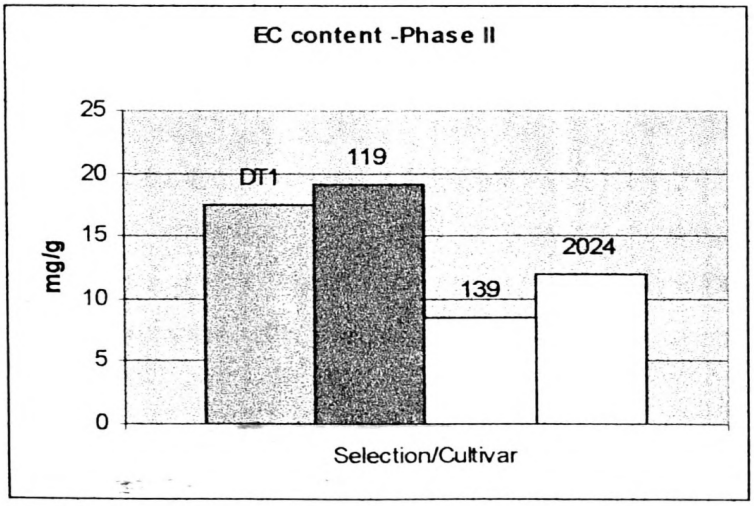

Figure 4.9: Comparison of EC content in phase II selections, TRI 2024 & DT1

4.2.4 Selections from phase II-EGCG content

Selections 118(55.58mg/g) and 139(59.41mg/g) gave significantly lower levels of EGCG content when compared with TRI 2024(Table 4.2). When we consider EGCG content together with EC content in the selection 118 it should fall in to the blister blight resistant category. Intrerstingly according to the findings made by the Pathology division selection 118 falls into the resistant category indicating a possible use of the levels of these two compounds as marker for blister blight resistance.

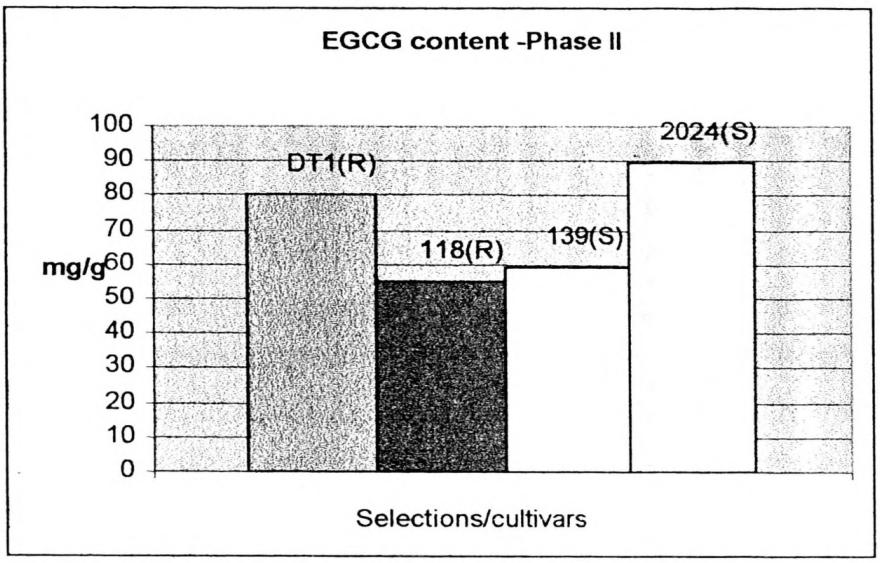

Figure 4.10: Comparison of EGCG content in phase II selections, TRI 2024 & DT1

4.2.5 Selections from phase II – ECG content

Selection 118(23.52mg/g), 48(25.84mg/g), 43(27.15mg/g) and 139(17.30mg/g) gave significantly lower levels for ECG when compared with TRI 2024(Table 4.2). The ECG levels obtained for selection 118 and 139 were significantly lower when compared with DT1 (Figure 4.11). These results indicate that ECG levels in the tea plant do not have any correlation with the blister blight disease resistant.

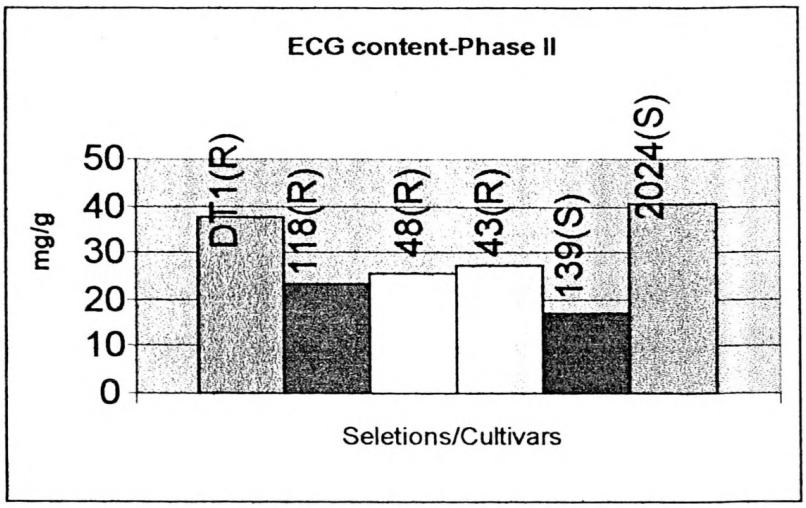

Figure 4.11: Comparison of ECG content in phase II selections, TRI 2024 & DT1

It is reported that flavan-3-ol (catechin) are directly or indirectly involved in the defense mechanism of blister blight through the formation of proanthocyanidins in the tea plant (Punyasiri et al., 2003). This study also suggested that the flavan-3-ols can be converted to fungitoxic proanthocyanidins as part of the defense mechanism. Studies conducted on other plants such as apple showed the involvement of flavan-3-ols in the resistant of apple against scab disease (Feucht and Treutter 1999). Feucht and Treutter (1999) also showed that the accumulation of catechin were induced by the action of the fungus *Vebturea inaequalis* in apple tissue indicating that flavan-3-ols could act as a barrier restricting the growth of the fungus

CHAPTER 05

5. CONCLUSION & FURTHER RECOMMENDATION

Conclusion

Based on the results of this project following conclusions can be made.

1. EC content & EGCG contents in the tea plant can be use as a possible chemical markers in order to select blister blight resistance.

2. EGC content & ECG content in the tea plant have no correlation with, blister blight disease resistance & susceptibility.

Further Recommendation

The identified chemical marker (EC & EGCG) should be monitored closely during phase I & phase II stages of the breeding programme. Once the final ratings of these selections on blister blight resistance are available (by the Plant Pathology Division) a more reliable correlation between the marker and blister blight disease resistance could be established.

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APPENDIX

Table 3.3 Consensus Relative Response Factors

Component	Relative Response Factor (RRF) with respect to caffeine (calculated on standard DM basis)
Gallic acid	0.84
(-)-Epigallocatechin (EGC)	11.24
(+)-Catechin ©	3.58
(-)-Epicatechin (EC)	3.67
(-)-Epigallocatechin gallate (EGCG)	1.72
(-)-Epicatechin gallate (ECG)	1.42

(Adapted from document :ISO/TC 34/SC 8 N 531)

Phase 1

The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
Sel	9	19 2024 27 31 DT1 Y5 Y6 Y6B Y8

Number of observations 27

The SAS System

The GLM Procedure

Dependent Variable: EGC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	2184.150933	273.018867	9.70	<.0001
Error	18	506.824733	28.156930		
Corrected Total	26	2690.975667			

R-Square	Coeff Var	Root MSE	EGC Mean
0.811658	15.06951	5.306310	35.21222

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sel	8	2184.150933	273.018867	9.70	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sel	8	2184.150933	273.018867	9.70	<.0001

The SAS System
The GLM Procedure

Dependent Variable: CF

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	282.3000296	35.2875037	2.89	0.0291
Error	18	219.4281333	12.1904519		
Corrected Total	26	501.7281630			

R-Square	Coeff Var	Root MSE	CF Mean
0.562655	9.123996	3.491483	38.26704

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sel	8	282.3000296	35.2875037	2.89	0.0291

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sel	8	282.3000296	35.2875037	2.89	0.0291

The SAS System
The GLM Procedure

Dependent Variable: EC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	149.9561185	18.7445148	3.17	0.0200
Error	18	106.4068000	5.9114889		
Corrected Total	26	256.3629185			

R-Square	Coeff Var	Root MSE	EC Mean
0.584937	16.66157	2.431355	14.59259

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sel	8	149.9561185	18.7445148	3.17	0.0200

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sel	8	149.9561185	18.7445148	3.17	0.0200

The SAS System
The GLM Procedure

Dependent Variable: EGCG

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	5817.022400	727.127800	12.63	<.0001
Error	18	1036.210200	57.567233		
Corrected Total	26	6853.232600			

R-Square	Coeff Var	Root MSE	EGCG Mean
0.848800	7.985799	7.587307	95.01000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sel	8	5817.022400	727.127800	12.63	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sel	8	5817.022400	727.127800	12.63	<.0001

The SAS System

The GLM Procedure

Dependent Variable: ECG

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	1853.751800	231.718975	21.86	<.0001
Error	18	190.783867	10.599104		
Corrected Total	26	2044.535667			

R-Square	Coeff Var	Root MSE	ECG Mean
0.906686	9.300314	3.255626	35.00556

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sel	8	1853.751800	231.718975	21.86	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sel	8	1853.751800	231.718975	21.86	<.0001

The SAS System
The GLM Procedure

Dunnett's t Tests for EGC

NOTE: This test controls the Type I experiment wise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	18
Error Mean Square	28.15693
Critical Value of Dunnett's t	2.93514
Minimum Significant Difference	12.717

Comparisons significant at the 0.05 level are indicated by ***.

Sel Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
19 - DT1	14.037	1.320	26.753	***
Y6B - DT1	13.797	1.080	26.513	***
Y6 - DT1	13.657	0.940	26.373	***
27 - DT1	10.200	-2.517	22.917	
31 - DT1	-0.213	-12.930	12.503	
Y5 - DT1	-4.737	-17.453	7.980	
Y8 - DT1	-7.580	-20.297	5.137	
2024 - DT1	-8.600	-21.317	4.117	

The SAS System

Dunnett's t Tests for CF

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	18
Error Mean Square	12.19045
Critical Value of Dunnett's t	2.93514
Minimum Significant Difference	8.3674

Comparisons significant at the 0.05 level are indicated by ***.

Sel Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
19 - DT1	6.713	-1.654	15.081
27 - DT1	5.187	-3.181	13.554
Y5 - DT1	1.770	-6.597	10.137
Y6B -DT1	0.137	-8.231	8.504
31 - DT1	-1.370	-9.737	6.997
2024 - DT1	-2.173	-10.541	6.194
Y8 - DT1	-2.597	-10.964	5.771
Y6 - DT1	-2.863	-11.231	5.504

The SAS System
The GLM Procedure

Dunnett's t Tests for EC

NOTE: This test controls the Type I experiment wise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	18
Error Mean Square	5.911489
Critical Value of Dunnett's t	2.93514
Minimum Significant Difference	5.8268

Comparisons significant at the 0.05 level are indicated by ***.

Sel Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
Y8 - DT1	-0.540	-6.367	5.287	
Y6 - DT1	-0.637	-6.463	5.190	
31 - DT1	-1.030	-6.857	4.797	
19 - DT1	-2.833	-8.660	2.993	
Y6B - DT1	-3.050	-8.877	2.777	
27 - DT1	-4.943	-10.770	0.883	
2024 - DT1	-5.460	-11.287	0.367	
Y5 - DT1	-6.983	-12.810	-1.157	***

The SAS System
The GLM Procedure

Dunnett's t Tests for EGCG

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	18
Error Mean Square	57.56723
Critical Value of Dunnett's t	2.93514
Minimum Significant Difference	18.183

Comparisons significant at the 0.05 level are indicated by ***.

Sel Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
Y5 - DT1	41.427	23.243	59.610	***
19 - DT1	32.170	13.987	50.353	***
27 - DT1	25.413	7.230	43.597	***
Y8 - DT1	21.103	2.920	39.287	***
2024 - DT1	9.143	-9.040	27.327	
Y6 - DT1	1.947	-16.237	20.130	
Y6B - DT1	1.467	-16.717	19.650	
31 - DT1	1.310	-16.873	19.493	

The SAS System
The GLM Procedure

Dunnett's t Tests for ECG

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	18
Error Mean Square	10.5991
Critical Value of Dunnett's t	2.93514
Minimum Significant Difference	7.8022

Comparisons significant at the 0.05 level are indicated by ***.

Sel Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
Y8 - DT1	14.940	7.138	22.742	***
2024 - DT1	2.703	-5.099	10.506	
Y5 - DT1	1.143	-6 659	8.946	
31 - DT1	-1.207	-9.009	6.596	
19 - DT1	-6.677	-14.479	1.126	
27 - DT1	-8.423	-16.226	-0.621	***
Y6 - DT1	-10.317	-18.119	-2.514	***
Y6B - DT1	-14.823	-22.626	-7.021	***

The SAS System
The GLM Procedure

Dunnett's t Tests for EGC

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	18
Error Mean Square	28.15693
Critical Value of Dunnett's t	2.93514
Minimum Significant Difference	12.717

Comparisons significant at the 0.05 level are indicated by ***.

Sel Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
19 - 2024	22.637	9.920 35.353	***
Y6B - 2024	22.397	9.680 35.113	***
Y6 - 2024	22.257	9.540 34.973	***
27 - 2024	18.800	6.083 31.517	***
DT1 - 2024	8.600	-4.117 21.317	
31 - 2024	8.387	-4.330 21.103	
Y5 - 2024	3.863	-8.853 16.580	
Y8 - 2024	1.020	-11.697 13.737	

The SAS System
The GLM Procedure

Dunnett's t Tests for CF

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	18
Error Mean Square	12.19045
Critical Value of Dunnett's t	2.93514
Minimum Significant Difference	8.3674

Comparisons significant at the 0.05 level are indicated by ***.

Sel Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
19 - 2024	8.887	0.519 17.254	***
27 - 2024	7.360	-1.007 15.727	
Y5 - 2024	3.943	-4.424 12.311	
Y6B - 2024	2.310	-6.057 10.677	
DT1 - 2024	2.173	-6.194 10.541	
31 - 2024	0.803	-7.564 9.171	
Y8 - 2024	-0.423	-8.791 7.944	
Y6 - 2024	-0.690	-9.057 7.677	

The SAS System
The GLM Procedure

Dunnett's t Tests for EC

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	18
Error Mean Square	5.911489
Critical Value of Dunnett's t	2.93514
Minimum Significant Difference	5.8268

Comparisons significant at the 0.05 level are indicated by ***.

Sel Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
DT1 - 2024	5.460	-0.367	11.287
Y8 - 2024	4.920	-0.907	10.747
Y6 - 2024	4.823	-1.003	10.650
31 - 2024	4.430	-1.397	10.257
19 - 2024	2.627	-3.200	8.453
Y6B - 2024	2.410	-3.417	8.237
27 - 2024	0.517	-5.310	6.343
Y5 - 2024	-1.523	-7.350	4.303

The SAS System
The GLM Procedure

Dunnett's t Tests for EGCG

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	18
Error Mean Square	57.56723
Critical Value of Dunnett's t	2.93514
Minimum Significant Difference	18.183

Comparisons significant at the 0.05 level are indicated by ***.

Sel Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
Y5 - 2024	32.283	14.100	50.467	***
19 - 2024	23.027	4.843	41.210	***
27 - 2024	16.270	-1.913	34.453	
Y8 - 2024	11.960	-6.223	30.143	
Y6 - 2024	-7.197	-25.380	10.987	
Y6B - 2024	-7.677	-25.860	10.507	
31 - 2024	-7.833	-26.017	10.350	
DT1 - 2024	-9.143	-27.327	9.040	

The SAS System
The GLM Procedure

Dunnett's t Tests for ECG

NOTE: This test controls the Type I experiment wise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	18
Error Mean Square	10.5991
Critical Value of Dunnett's t	2.93514
Minimum Significant Difference	7.8022

Comparisons significant at the 0.05 level are indicated by ***.

Sel Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
Y8 - 2024	12.237	4.434	20.039	***
Y5 - 2024	-1.560	-9.362	6.242	
DT1 - 2024	-2.703	-10.506	5.099	
31 - 2024	-3.910	-11.712	3.892	
19 - 2024	-9.380	-17.182	-1.578	***
27 - 2024	-11.127	-18.929	-3.324	***
Y6 - 2024	-13.020	-20.822	-5.218	***
Y6B - 2024	-17.527	-25.329	-9.724	***

Phase II
The SAS System
The GLM Procedure

Class Level Information

Class	Levels	Values
Sel	11	118 119 139 2 2024 24 43 48 58 61 DT1

Number of observations 33

Dependent Variables With Equivalent Missing Value Patterns

Pattern	Obs	Dependent Variables
1	32	EGC
2	33	CF EC EGCG ECG

NOTE: Variables in each group are consistent with respect to the presence or absence of missing values.

The SAS System
The GLM Procedure

Dependent Variable: EGC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	2237.331017	223.733102	7.85	<.0001
Error	21	598.828183	28.515628		
Corrected Total	31	2836.159200			

R-Square	Coeff Var	Root MSE	EGC Mean
0.788859	19.23459	5.340003	27.76250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sel	10	2237.331017	223.733102	7.85	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sel	10	2237.331017	223.733102	7.85	<.0001

The SAS System
The GLM Procedure

Dunnett's t Tests for EGC

NOTE: This test controls the Type I experiment wise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	21
Error Mean Square	28.51563
Critical Value of Dunnett's t	2.97466

Comparisons significant at the 0.05 level are indicated by ***.

Sel Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
119 - DT1	9.487	-3.483	22.456	
48 - DT1	8.597	-4.373	21.566	
24 - DT1	4.238	-10.262	18.739	
2 - DT1	-2.503	-15.473	10.466	
118 - DT1	-5.200	-18.170	7.770	
139 - DT1	-7.417	-20.386	5.553	
43 - DT1	-8.447	-21.416	4.523	
2024 - DT1	-8.600	-21.570	4.370	
58 - DT1	-15.873	-28.843	-2.904	***
61 - DT1	-16.113	-29.083	-3.144	***

The SAS System

The GLM Procedure

Dunnett's t Tests for EGC

NOTE: This test controls the Type I experiment wise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	21
Error Mean Square	28.51563
Critical Value of Dunnett's t	2.97466

Comparisons significant at the 0.05 level are indicated by ***.

Sel Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
119 - 2024	18.087	5.117	31.056	***
48 - 2024	17.197	4.227	30.166	***
24 - 2024	12.838	-1.662	27.339	
DT1 - 2024	8.600	-4.370	21.570	
2 - 2024	6.097	-6.873	19.066	
118 - 2024	3.400	-9.570	16.370	
139 - 2024	1.183	-11.786	14.153	
43 - 2024	0.153	-12.816	13.123	
58 - 2024	-7.273	-20.243	5.696	
61 - 2024	-7.513	-20.483	5.456	

The SAS System
The GLM Procedure

Dependent Variable: CF

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	434.1182303	43.4118230	1.89	0.1023
Error	22	504.8956667	22.9498030		
Corrected Total	32	939.0138970			

R-Square	Coeff Var	Root MSE	CF Mean
0.462313	14.55679	4.790595	32.90970

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sel	10	434.1182303	43.4118230	1.89	0.1023

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sel	10	434.1182303	43.4118230	1.89	0.1023

The SAS System
The GLM Procedure

Dependent Variable: EC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	350.1341515	35.0134152	4.48	0.0016
Error	22	171.9876000	7.8176182		
Corrected Total	32	522.1217515			

R-Square	Coeff Var	Root MSE	EC Mean
0.670599	19.41830	2.796000	14.39879

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sel	10	350.1341515	35.0134152	4.48	0.0016

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sel	10	350.1341515	35.0134152	4.48	0.0016

The SAS System
The GLM Procedure

Dependent Variable: EGCG

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	3435.704630	343.570463	2.49	0.0359
Error	22	3039.249133	138.147688		
Corrected Total	32	6474.953764			

R-Square	Coeff Var	Root MSE	EGCG Mean
0.530615	15.82844	11.75362	74.25636

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sel	10	3435.704630	343.570463	2.49	0.0359

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sel	10	3435.704630	343.570463	2.49	0.0359

The SAS System
The GLM Procedure

Dependent Variable: ECG

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	2350.576824	235.057682	9.96	<.0001
Error	22	519.431400	23.610518		
Corrected Total	32	2870.008224			

R-Square	Coeff Var	Root MSE	ECG Mean
0.819014	14.95794	4.859066	32.48485

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sel	10	2350.576824	235.057682	9.96	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sel	10	2350.576824	235.057682	9.96	<.0001

The SAS System

The GLM Procedure

Dunnett's t Tests for CF

NOTE: This test controls the Type I experiment wise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	22
Error Mean Square	22.9498
Critical Value of Dunnett's t	2.95715
Minimum Significant Difference	11.567

Comparisons significant at the 0.05 level are indicated by ***.

Sel Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
119 - DT1	-0.043	-11.610	11.524
2 - DT1	-2.107	-13.674	9.460
2024 - DT1	-2.173	-13.740	9.394
48 - DT1	-2.883	-14.450	8.684
58 - DT1	-4.013	-15.580	7.554
43 - DT1	-6.433	-18.000	5.134
61 - DT1	-7.043	-18.610	4.524
24 - DT1	-7.223	-18.790	4.344
139 - DT1	-10.100	-21.667	1.467
118 - DT1	-11.040	-22.607	0.527

The SAS System
The GLM Procedure

Dunnett's t Tests for EC

NOTE: This test controls the Type I experiment wise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	22
Error Mean Square	7.817618
Critical Value of Dunnett's t	2.95715
Minimum Significant Difference	6.7509

Comparisons significant at the 0.05 level are indicated by ***.

Sel Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
119 - DT1	1.733	-5.018	8.484	
2 - DT1	0.533	-6.218	7.284	
48 - DT1	-0.060	-6.811	6.691	
24 - DT1	-1.250	-8.001	5.501	
118 - DT1	-3.317	-10.068	3.434	
58 - DT1	-4.810	-11.561	1.941	
2024 - DT1	-5.473	-12.224	1.278	
43 - DT1	-5.517	-12.268	1.234	
61 - DT1	-6.177	-12.928	0.574	
139 - DT1	-8.933	-15.684	-2.182	***

The SAS System
The GLM Procedure

Dunnett's t Tests for EGCG

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	22
Error Mean Square	138.1477
Critical Value of Dunnett's t	2.95715
Minimum Significant Difference	28.379

Comparisons significant at the 0.05 level are indicated by ***.

Sel Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
2024 - DT1	9.143	-19.236	37.522
61 - DT1	5.427	-22.952	33.806
2 - DT1	1.753	-26.626	30.132
58 - DT1	1.453	-26.926	29.832
48 - DT1	-5.350	-33.729	23.029
24 - DT1	-8.030	-36.409	20.349
43 - DT1	-11.177	-39.556	17.202
119 - DT1	-12.503	-40.882	15.876
139 - DT1	-20.710	-49.089	7.669
118 - DT1	-24.543	-52.922	3.836

The SAS System
The GLM Procedure

Dunnett's t Tests for ECG

NOTE: This test controls the Type I experiment wise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	22
Error Mean Square	23.61052
Critical Value of Dunnett's t	2.95715
Minimum Significant Difference	11.732

Comparisons significant at the 0.05 level are indicated by ***.

Sel Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
61 - DT1	8.010	-3.722	19.742	
58 - DT1	3.487	-8.246	15.219	
2024 - DT1	2.703	-9.029	14.436	
2 - DT1	2.403	-9.329	14.136	
119 - DT1	-7.477	-19.209	4.256	
24 - DT1	-8.270	-20.002	3.462	
43 - DT1	-10.377	-22.109	1.356	
48 - DT1	-11.680	-23.412	0.052	
118 - DT1	-14.000	-25.732	-2.268	***
139 - DT1	-20.223	-31.956	-8.491	***

The SAS System
The GLM Procedure

Dunnett's t Tests for CF

NOTE: This test controls the Type I experiment wise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	22
Error Mean Square	22.9498
Critical Value of Dunnett's t	2.95715
Minimum Significant Difference	11.567

Comparisons significant at the 0.05 level are indicated by ***.

Sel Comparison	Difference Between Means	Simultaneous 95% Confidence Limits
DT1 - 2024	2.173	-9.394 13.740
119 - 2024	2.130	-9.437 13.697
2 - 2024	0.067	-11.500 11.634
48 - 2024	-0.710	-12.277 10.857
58 - 2024	-1.840	-13.407 9.727
43 - 2024	-4.260	-15.827 7.307
61 - 2024	-4.870	-16.437 6.697
24 - 2024	-5.050	-16.617 6.517
139 - 2024	-7.927	-19.494 3.640
118 - 2024	-8.867	-20.434 2.700

The SAS System
The GLM Procedure

Dunnett's t Tests for EC

NOTE: This test controls the Type I experiment wise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	22
Error Mean Square	7.817618
Critical Value of Dunnett's t	2.95715
Minimum Significant Difference	6.7509

Comparisons significant at the 0.05 level are indicated by ***.

Sel Comparison	Difference Between Means	Simultaneous 95% Confidence Limits
119 - 2024	7.207	0.456 13.958 ***
2 - 2024	6.007	-0.744 12.758
DT1 - 2024	5.473	-1.278 12.224
48 - 2024	5.413	-1.338 12.164
24 - 2024	4.223	-2.528 10.974
118 - 2024	2.157	-4.594 8.908
58 - 2024	0.663	-6.088 7.414
43 - 2024	-0.043	-6.794 6.708
61 - 2024	-0.703	-7.454 6.048
139 - 2024	-3.460	10.211 3.291

The SAS System
The GLM Procedure

Dunnett's t Tests for EGCG

NOTE: This test controls the Type I experiment wise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	22
Error Mean Square	138.1477
Critical Value of Dunnett's t	2.95715
Minimum Significant Difference	28.379

Comparisons significant at the 0.05 level are indicated by ***.

Sel Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		
61 - 2024	-3.717	-32.096	24.662	
2 - 2024	-7.390	-35.769	20.989	
58 - 2024	-7.690	-36.069	20.689	
DT1 - 2024	-9.143	-37.522	19.236	
48 - 2024	-14.493	-42.872	13.886	
24 - 2024	-17.173	-45.552	11.206	
43 - 2024	-20.320	-48.699	8.059	
119 - 2024	-21.647	-50.026	6.732	
139 - 2024	-29.853	-58.232	-1.474	***
118 - 2024	-33.687	-62.066	-5.308	***

The SAS System
The GLM Procedure

Dunnett's t Tests for ECG

NOTE: This test controls the Type I experiment wise error for comparisons of all treatments against a control.

Alpha	0.05
Error Degrees of Freedom	22
Error Mean Square	23.61052
Critical Value of Dunnett's t	2.95715
Minimum Significant Difference	11.732

Comparisons significant at the 0.05 level are indicated by ***.

Sel Comparison	Difference Between Means	Simultaneous 95% Confidence Limits	
61 - 2024	5.307	-6.426 17.039	
58 - 2024	0.783	-10.949 12.516	
2 - 2024	-0.300	-12.032 11.432	
DT1 - 2024	-2.703	-14.436 9.029	
119 - 2024	-10.180	-21.912 1.552	
24 - 2024	-10.973	-22.706 0.759	
43 - 2024	-13.080	-24.812 -1.348	***
48 - 2024	-14.383	-26.116 -2.651	***
118 - 2024	-16.703	-28.436 -4.971	***
139 - 2024	-22.927	-34.659 -11.194	***

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